

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 2003/000619

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/465

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, PAJ, MEDLINE, BIOSIS, EMBASE, EBI, CHEM.ABS.DATA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	The Journal of Biological Chemistry, Volume 278, no. 4, 2003, Henri R. Nordlund et al: "Enhancing the Thermal Stability of Avidin", page 2479 - page 2483 --	1-16,19-21
X	Nature Biotechnology, Volume 14, 1996, Gabriel O. Reznik et al: "Streptavidins with intersubunit crosslinks have enhanced stability", page 1007 - page 1011 --	1-16,19-21
X	WO 9711183 A1 (TRUSTEES OF BOSTON UNIVERSITY), 27 March 1997 (27.03.1997), figure 17, claim 37 --	1-16,19-21

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

21 January 2004

Date of mailing of the international search report

28.01.2004

Name and mailing address of the ISA/

Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

Terese Persson/BS

Telephone No. +46 8 782 25 00

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FEBS Letters, Volume 467, 2000, Ari T. Marttila et al: "Recombinant Neutralite Avidin: a non-glycosylated, acidic mutant of chicken avidin that exhibits high affinity for biotin and low non-specific binding properties", page 31 - page 36, the abstract; page 32, table 1; page 35, column 2, paragraph 3 --	1-2,17,21
X	Biochem. J. Volume 363, 2002, Olli H. Laitinen et al: "Chicken avidin-related proteins show altered biotin-binding and physico-chemical properties as compared with avidin", page 609 - page 617, abstract, figure 1; page 615, left column, second paragraph --	1-2,13,15,18,21
X	WO 0105977 A1 (YEDA RESEARCH AND DEVELOPMENT CO. LTD.), 25 January 2001 (25.01.2001), figure 3 --	1-2,14,21
A	Nature, Volume 342, 16 November 1989, Masazumi Matsumura et al: "Substantial increase of protein stability by multiple disulphide bonds", page 291 - page 293 --	1-16,18-21
A	The Journal of Biological Chemistry, Volume 263, No. 24, 1988, C. Nick Pace et al, "Conformational Stability and Activity of Ribonuclease T1 with Zero, One, and Two Intact Disulfide Bonds*", pages 11820-11825 --	1-16,18-21
A	BioEssays, Volume 8, No. 2, 1988, Thomas E. Creighton, "Disulphide Bonds and Protein Stability", pages 57-63 --	1-16,18-21
A	Applied Biochemistry and Biotechnology, Volume 53, 1995, Edward A. Bayer et al, "Preparation of Deglycosylated Egg White Avidin", pages 1-9 -- -----	17

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/FI 2003/00619**

**Box No. I** Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

- ☒ a sequence listing  
☐ table(s) related to the sequence listing

b. format of material

- ☒ in written format  
☒ in computer readable form

c. time of filing/furnishing

- ☒ contained in the international application as filed  
☐ filed together with the international application in computer readable form  
☒ furnished subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/FI 2003/00619**

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**se extra sheet**

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

This International Search Authority (ISA) considers that there are 3 inventions covered by the claims indicated as follows:

1.1 Claims 1-2 (partly), 3-12, 13 (partly), 16, 19-21 (partly) are directed to a mutant of a biotin binding protein wherein the mutant has intermonomeric disulphide bridges in the tetramer. The mutants have increased thermostability compared to the wild type biotin binding protein.

1.2 Claims 15, 21 (partly) are directed to a thermally stable AVR4/5.

2. Claims 1-2 (partly), 13 (partly), 14, 18-21 (partly) are directed to a mutant of a biotin binding protein wherein a cysteine/ intramonomeric disulphide bridges have been substituted/deleted.

3. Claim 17, 18 (partly), 21 (partly) are directed to a mutant of a biotin binding protein wherein asparagine 43 (glycosylation site) of AVR4/5 have been changed to glutamic acid.

The present application has been considered to contain 3 inventions which are not linked such that they form a single general inventive concept, as required by Rules 13.1, 13.2 and 13.3 PCT for the following reasons:

The prior art is represented by Reznik GO et al., Nature Biotechnology, Volume 14, August 1996, pages 1007-1011 (D1). D1 concerns streptavidin (a biotin binding protein) with intersubunit crosslinks. In one mutant, His at position 127 has been substituted by Cys, forming an intersubunit disulphide bond (Stv-C127). Stv-C127 shows enhanced thermal stability compared to a natural core streptavidin (see Figure 5). The formation of the disulphide bond had no effect on the biotin-binding ability (page 1008, left column, second paragraph). Thus, the concept of the present invention, i.e. to provide a mutant biotin binding protein having an improved thermal stability compared to the wild type, by forming intermonomeric disulphide bridges is already known in the prior art. Therefore, no unifying special technical feature has been found between the different inventions listed above, that is, between the different solutions of providing biotin binding proteins having improved properties compared to the wild type protein.

Invention 1.1 relates to the problem of providing a mutant biotin binding protein having an enhanced thermal stability compared to the wild type protein. This problem appears to be solved by introducing (substituting for) cysteine residues in order to form intermonomeric disulphide bridges.

Invention 1.2 relates to the problem of providing thermally stable AVR4/5 in general.

Invention 2 relates to the problem of providing a mutant biotin binding protein wherein naturally occurring cysteine residues have been substituted/deleted. The problem appears to be solved by changing cysteine 60 in AVR1, AVR3, AVR6 or AVR7 or by deleting the four intramonomeric disulphide bridges in chicken avidin. It seems to be unclear which the improved properties compared to the wild type protein are.

.../...

Invention 3 relates to the problem of providing a mutant biotin binding protein having an altered glycosylation. The problem appears to be solved by substituting asparagine 43 in AVR4/5 for glutamic acid. It seems to be unclear which the improved properties compared to the wild type protein are, except for the known fact that deglycosylation may decrease non-specific binding. In the description (page 15, lines 22-24) it says that the glycosylation of Asn-43 had no marked influence on biotin binding, thermal stability or structural properties of AVR4/5.

In conclusion, therefore, the 3 group of claims are not linked by common or corresponding technical features and define different inventions not linked by a single general inventive concept. The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

24/12/2003

International application No.

PCT/FI 2003/000619

WO	9711183	A1	27/03/1997	AU	5917796	A	09/04/1997
				CA	2222035	A	27/03/1997
				EP	0856055	A	05/08/1998
				US	6022951	A	08/02/2000
				US	6207390	B	27/03/2001
-----							
WO	0105977	A1	25/01/2001	AU	6344100	A	05/02/2001
-----							